

BRIEF PAPERS

THE PHYSICAL NATURE OF TRANSPIRATIONAL PULL¹

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Though there seems to be rather general agreement (with few exceptions²) among plant physiologists that the Cohesion Theory of the ascent of sap best agrees with the known facts, the nature of the initiating force is not so generally agreed upon. Consequently, to be on the safe side, it is usually referred to as the "transpirational pull," without attempting to explain the physical basis of this "pull." In most modern texts, an attempt has been made to relate it to diffusion. Thus, according to Meyer and Anderson (6):

Within the lamina of the leaf, gradients of diffusion pressure deficits, gradually increasing in magnitude from cell to cell in the direction in which water is moving are established between the xylem ducts and the cells from which evaporation is occurring. Water therefore moves from a given vessel or tracheid into adjacent cells, which results in the development of a tension in the water column occupying that element of the xylem.

The same concept, in different words, is to be found in Curtis and Clark (4), Bonner and Galston (2), and even Maximov (5). This implies that the transpirational pull really involves a push in the top fraction of a millimeter of the column (from the top of the xylem through the living cells, to the evaporation surface of the mesophyll cells) which initiates a pull below it. Thus the cohesive force would be responsible for the rise of the whole column except this uppermost fraction of a millimeter.

This would also mean that the cohesive pull is initiated not at the water surface, but somewhere back of this—in other words, instead of raising itself by hanging on to the inner surface of the cell wall, the column would have to pull itself up by its own bootstraps! It is difficult to understand the popularity of this theory. It certainly does not follow the principle of Occam's razor since it replaces a simple concept by a complex one. Furthermore, the Askenasy experiment cannot be explained in this way since no living cells are involved. In this case, at least, the pull must be initiated right at the surface, rather than a fraction of a millimeter below, a fact that is recognized by Meyer and Anderson.

The usual point of view seems to be that if the diffusion gradient is in the right direction, then diffusion must account for whatever flow that occurs. Actually, what must be demonstrated is that the *rate of diffusion of the water in the liquid state from the vessels to the mesophyll cells is adequate to keep up with the rate of diffusion of the water vapor from the mesophyll cells into the air*. This point can be

cleared up by a consideration of Fick's law of diffusion:

$$\frac{s}{t} = Da \frac{C_1 - C_2}{x}$$

where s = amount of substance diffusing

t = time

D = coefficient of diffusion

a = area across which diffusion is occurring

C₁ = higher concentration

C₂ = lower concentration

x = distance separating C₁ from C₂

The question now is, whether or not the ratio s/t for the diffusing water vapor is of the same order as s/t for the diffusing liquid water—i.e., it is required to prove that

$$\frac{s/t \text{ (water vapor)}}{s/t \text{ (liquid water)}}$$

is of the order of 1 in a transpiring leaf, in which rate of water loss from the leaf is just compensated by rate of water intake.

Let us assume a commonly found set of conditions: a turgid plant with open stomata in an atmosphere of 60 % relative humidity, the mesophyll cells having an osmotic potential of 15 atms and maintaining their turgor unchanged (i.e., rate of water absorption by mesophyll cells = rate of water loss). The problem can be broken down into four parts.

a) What is the ratio

$$\frac{D \text{ (water vapor)}}{D \text{ (liquid water)}}?$$

The coefficient of diffusion of water vapor is 0.22 cm²/sec, the value for liquid water is of the order of 2.2 × 10⁻⁵ cm²/sec, assuming that it is about the same as that for the physically similar HCl in water (see International Critical Tables). Therefore

$$\frac{D \text{ (water vapor)}}{D \text{ (liquid H}_2\text{O)}} = 10^4$$

b) What is the ratio

$$\frac{C_1 - C_2 \text{ (water vapor)}}{C_1 - C_2 \text{ (liquid water)}}?$$

The actual concentrations of the liquid and gaseous water cannot be determined, but the quantity that is more important in diffusion—the activities—can be indirectly measured by means of the osmotic quantities which are related to the activities of the water molecules.

Since the mesophyll cells have an osmotic potential of 15 atms, their relative humidity is about 99 %, or 39 % above that of the atmosphere. C₁ - C₂ (water

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² See Scholander et al (9).

vapor) is therefore about 600 atms (since 1 % r.h. is equivalent to about 15 atms). Since the mesophyll cells are turgid, $C_1 - C_2$ (liquid water) is less than the difference between the osmotic potential of the mesophyll cells and that of the vessel sap. It therefore cannot be more than about 10 atms. Therefore

$$\frac{C_1 - C_2 \text{ (water vapor)}}{C_1 - C_2 \text{ (liquid water)}} = \frac{600}{10} = 60$$

c) What is the ratio

$$\frac{x \text{ (water vapor)}}{x \text{ (liquid water)}} ?$$

Since C_1 (water vapor) is taken as the concentration at the surface of the mesophyll cells and C_2 as that in the external atmosphere, x (water vapor) will be the shortest distance between the mesophyll cells and the external atmosphere. This must be the distance from the surface of the mesophyll cells adjacent to the substomatal cavity to the outer surface of the leaf (or a little above it). Again, since C_1 (liquid water) is that of the vessel sap, and C_2 (liquid water) that of these same mesophyll cells adjacent to the substomatal cavity, x (liquid water) will be the distance across the cells between these two—a distance at least as great as the above which is equivalent to about two cells. Therefore

$$\frac{x \text{ (water vapor)}}{x \text{ (liquid water)}} = 1 \text{ (app.)}$$

d) What is

$$\frac{a \text{ (water vapor)}}{a \text{ (liquid water)}} ?$$

Since a is the area across which diffusion is occurring, a (water vapor) for a single leaf must be equal to the total stomatal area of that leaf and a (liquid water) the total area of the vessels in the leaf at right angles to the direction of flow to the stomata. Since, however, we are dealing with small apertures in both cases, the diffusion rate is proportional to the perimeters rather than the areas. This means that the effective area for diffusion of the water vapor is the whole surface of the leaf. Since the effective area for diffusion of the liquid water cannot be any greater than this, the ratio

$$\frac{a \text{ (water vapor)}}{a \text{ (liquid water)}} = 1 \text{ (at the least)}$$

Therefore, from a), b), c), and d),

$$\frac{s/t \text{ (water vapor)}}{s/t \text{ (liquid water)}} = 10^4 \times 60 \times 1 \times 1 = 6 \times 10^5$$

We are forced to conclude, then, that the rate of diffusion of water vapor from the leaf into an atmosphere of 60 % relative humidity is at the very least $600,000 \times$ the rate of diffusion of the liquid water from the vessels to the mesophyll cells in a normal, turgid, leaf with open stomata and with an osmotic potential of 15 atms in the mesophyll cells. And this does not take into account the retarding effect of the plasma

membrane on the diffusion of water through the living cells.

The diffusion of water in the liquid state is obviously far too slow to play any role whatever in the ascent of sap from the vascular stream to the mesophyll cell surface. Perhaps the main reason for suggesting this concept is the idea that surface forces are inadequate. Thus Preston (8) states:

No mention need therefore be made here either of the conception of atmospheric pressure as the driving pressure (since trees are often higher than the barometer column in water) or of surface tension.

Even physics texts sometimes give the same impression. In his discussion of capillarity Stewart (11) states:

Strictly speaking, the water is not lifted by the surface film. The upward force of the film causes a decrease in the pressure under the surface; above the surface the pressure is that of the atmosphere. Hence in the liquid under the film the pressure will be less than the atmospheric pressure, and the liquid will be forced up by the pressure on the outside of the tube.

On this basis, the maximum height attainable due to capillarity is 30 ft (i.e., that due to atmospheric pressure). That surface forces are able to exceed this has long been known from the Askenasy experiment. In fact, if this concept were correct, no capillary rise could occur in a vacuum (a point that is very easily disproved).

Similarly, Adam (1) states that

The liquid is not pulled up the tube by a hypothetical surface tension pulling on the walls, as is suggested by the explanation found in so many elementary textbooks—it has never been made clear what is the hook on the wall to which this "surface tension" attaches itself, nor how the hook contrives to move up the tube in advance of the rising meniscus. . . . the pressure difference follows from the free energy resident in the surface, and the liquid then flows up the tube under the hydrostatic pressure.

Older physics texts do seem to recognize that surface tension may cause a capillary rise above that due to atmospheric pressure alone. Thus, Millikan (7) states:

Hence, unless the ratio of the cohesion to the adhesion exceeds a certain limit, a thin film of the liquid must spread indefinitely over the surface of the solid.

Perhaps the only modern physiology text that considers the transpirational pull to be a surface force is that by Thomas (12). Crafts et al (3) clearly connect the two.

As Millikan points out, the capillary force is dependent on and even initiated by the adhesion between the liquid and the walls of the capillary tube. In the case of the evaporating surface of the mesophyll cells, this adhesive force would be the imbibitional force of the cell wall. That this force is adequate to account for the ascent of sap is obvious from the fact that imbibition pressures of as high as about 1000 atms have been measured (Shull, 10). Furthermore, this

pressure rises rapidly with dehydration of the cell wall, and would therefore increase the tension as the transpiration exceeded the absorption rate.

Thus a consistent concept of the Cohesion Theory requires that the initiating (adhesive or imbibitional) force is located at the evaporating surface and that the cohesive force is transmitted from here all the way back through the living leaf cells to the vessel, down the vessels to the living root cells, all the way to the soil particle in contact with the absorbing root surface.

The process can be visualized on a molecular basis something as follows. The cell wall particles are all hydrated. If some water molecules evaporate from the particles at the outer surface of the mesophyll cell walls, these particles will attract water with a greater adsorptive force than the ones directly below them and will therefore adsorb some water molecules from them. Due to the cohesion of the water molecules, this will result in a rise of the column as a whole (if the force is large enough to overcome the gravitational and frictional forces).

Curtis and Clark (4) have objected to this concept from another point of view. By use of Poiseuille's Law, they have concluded that the force required to move water through the pores in the cell wall would require 100,000 atms for pores 0.1μ in diameter. That their conclusion is in error can be demonstrated by use of a Seitz filter. With less than an atmosphere pressure, it is possible to obtain a flow through one of these filters at least 100 times as rapid as that due to transpiration from a leaf of equal area, though the pores are small enough to hold back organisms of about 0.1μ in diameter.

Curtis and Clark's reasoning is based on the "assumption that a head of water of 1 m supplies water fast enough to supply the leaves when the vessels are 0.1 mm in diameter." In other words, they are assuming that the capillaries extend the whole length of the plant. But, if the limiting capillaries are simply those in the wall of a mesophyll cell, the length may be the thickness of the wall or about 1μ —i.e., 10^{-7} times the length of the vessels in a plant 10 m high. Since the $P \propto L$, the minimum pressure needed to initiate a flow through 0.1μ pores in a mesophyll cell wall would be only 0.01 atm instead of 100,000 atms.

The calculations for a leaf can be readily made. According to Poiseuille's Law:

$$P = \frac{8Vnl}{\pi r^4}$$

where P = difference in pressure at the two ends of the tube

V = volume flowing out of tube in unit time

n = 0.01 poise for water

l = length of the tube

r = radius of the tube

The main basis of Curtis and Clark's argument is that $P \propto (l/r^4)$. But this is counteracted by the extremely small value of V for a single pore (or, put in

another way, the very large number of pores involved). Thus V = volume moving through 1 pore

$$= \frac{\text{volume moving through 1 leaf}}{\text{number of pores per leaf}}$$

Let us assume a) the extraordinary rate of unity for transpiration ($1 \text{ ml/cm}^3 \times \text{hr}$). In cgs units this becomes $(1/3600 \text{ ml})/(\text{cm}^3 \times \text{sec})$. Let us also assume b) that the number of pores per leaf = total cell surface/2 \times the area of a single pore (i.e., that half the cell surface is pores), and c) that the cells are spherical. Actually the cells are irregular and have much more specific surface than this, but the error will be approximately canceled by the fact that the cells are in contact for part of their surface and, therefore, the whole surface is not available for evaporation.

From the above assumptions, the total cell surface = leaf volume times specific cell surface

$$= \frac{3 \times \text{leaf vol}}{r}$$

The number of pores (assumption b), if the area of one pore is $1 \mu^2$ or 10^{-8} cm^2 ,

$$= \frac{3 \times \text{leaf vol}}{r} \times \frac{1}{2 \times 10^{-8}} = \frac{1.5}{10^{-8} r}$$

$\therefore V$ (moving through 1 pore)

$$= \frac{1/3600}{1.5/10^{-8} r} = \frac{10^{-8} r}{5400} \text{ ml/sec}$$

If we assume the length of the pore = 10 times the radius (e.g., 1μ long and 0.1μ radius),

$$P = \frac{8 \times (10^{-8} r/5400) \times 10^{-2} \times 10 r}{\pi r^4} \\ = \frac{0.5 \times 10^{-12}}{r^2} \text{ dynes/cm}^2$$

If the pores are 0.1μ in radius (half the radius assumed by Curtis and Clark,

$$P = \frac{0.5 \times 10^{-12}}{(10^{-5})^2} \\ = 0.5 \times 10^{-2} \text{ dynes/cm}^2 \\ = 0.5 \times 10^{-8} \text{ atms.}$$

This value is, of course, only an approximation based on assumptions that greatly simplify the situation. However, the errors tend to cancel each other out and even if the assumptions led to an appreciable error, the value would still be insignificant. Even rough calculations readily reveal that the pressure required to produce the flow must be insignificant, for pure water moving under its own weight flows through the finest filter paper at a much more rapid rate per unit area than its most rapid loss from a leaf by transpiration—even when it covers the filter paper by a layer only 1 mm thick. Since the volume of water on each cm^2 is then only 0.1 ml, the pressure due to the weight of the water is $100 \text{ dynes/cm}^2 = 10^{-4} \text{ atm}$. Yet the pores of the finest filter paper are small

enough to hold back the smallest bacteria, and the distance l is at least 100 times greater than that of the cell wall's pores.

SUMMARY

1. Physiology texts state that the "transpirational pull" initiates the ascent of sap by means of a diffusion of liquid water from the top of the vessels in the leaf to the evaporating surface of the mesophyll cells. This concept is shown to be impossible because under commonly found conditions, the diffusion rate of the water vapor may be 600,000 times that of the liquid water.

2. The objection of Curtis and Clark that it would require a pressure of 100,000 atms to produce a flow through the microcapillaries of the cell wall is also shown to be in error. Actually, less than 0.5×10^{-8} atms would be needed.

3. A consistent and physically sound theory of the ascent of sap is possible only if it is assumed that surface tension forces initiate the rise—i.e., that the adhesive (imbibitional) forces are increased at the surface due to evaporation and this causes the rise of the whole column due to cohesion between the water molecules.

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EFFECT OF RIBONUCLEASE ON SALT ABSORPTION BY EXCISED MUNG BEAN ROOTS¹

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The transport of ions into plant root cells is generally believed to be by means of ion-binding carrier compounds. Several types of compounds have been proposed as carriers, but none has won universal support (6). Recently, from observations on the effect of ribonuclease on absorption of calcium by *Elodea* cells and from cytological evidence, Lansing and Rosenthal (4) have suggested that ribonucleic acid may function as an ion-binding carrier compound during salt absorption. The results reported here lend support to their proposal.

In this study, mung bean (*Phaseolus aureus*) were germinated and grown at 25° C with roots in an aerated 10^{-4} M CaCl_2 solution which was changed daily. The first centimeter of root tip was excised from 3-day-old seedlings. Fifty root tips were placed in a beaker, washed, and treated with a solution of crystalline ribonuclease (100 $\mu\text{gm/ml}$) for short periods at 25° C. The enzyme was preheated at 70° C for 20 minutes before use. Control roots were similarly treated but without the enzyme. After enzymic treat-

ment the roots were washed and placed in a 10^{-4} M solution of either RbCl with Rb^{86} or KH_2PO_4 with P^{32} . The activities of the solutions were less than 5 $\mu\text{c/l}$. In some cases the solutions contained $\text{Ca}(\text{NO}_3)_2$ of 10^{-3} M. The roots were allowed to absorb Rb or phosphate for 30 min at 25° C under vigorous aeration. After the absorption period, the roots were washed several times with inactive salt solution of 10^{-2} M and water and dried at 100° C. The radioactivity of the roots was determined in the conventional manner.

Typical results showing the effect of ribonuclease on Rb and phosphate absorption are presented in table I. The data are the means of duplicate determinations. The experiment has been repeated several times with similar results. The data indicate that there was a very marked effect of the enzyme on Rb absorption by mung bean roots. The enzymic effect was apparently influenced strongly by the presence of Ca in the absorption medium. In the absence of Ca in the absorption medium, the ribonuclease pretreatment enhanced the uptake of Rb by roots. The absorption was linear up to one hour. Rb uptake in the

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